CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

PARAQUAT DICHLORIDE

Chemical Code # 001601, Tolerance # 00205
SB 950 # 019

April 29, 1986
Revised 8/25, 9/15 & 11/24/86; 8/28/87; 7/14/89; 8/27/91; 6/01/92; 10/6/93; 8/11/04

I. DATA GAP STATUS

Chronic rat:	No data gap, possible adverse effects
Chronic dog:	No data gap, possible adverse effect
Oncogenicity rat:	No data gap, possible adverse effects
Oncogenicity mouse:	No data gap, possible adverse effect (not oncogenicity)
Reproduction rat:	No data gap, no adverse effect
Teratology rat:	No data gap, no adverse effect
Teratology mouse:	No Data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	Not required at this time

Note, Toxicology one-liners are attached
All record numbers through 212137 (volume 238) were examined.

** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T040811
Revised/updated by C. Aldous and J. Gee, 6/01/92; P. Iyer 10/6/93; Gee, 8/11/04

Note: the following pages contain summaries only. Each individual worksheet may contain additional effects.

II. TOXICOLOGY ONE LINERS AND DISCUSSION

COMBINED, RAT

NOTE: The "Second Peer Review of Paraquat" submitted by Reto Engler of Toxicology Branch/HED to Project Manager, Robert Taylor, dated 7/28/88, discusses primarily the Life Sciences Research (LSR) study below, which begins in CDFA records with volume 055. This Peer Review, reproduced in Appendix 1 of CDFA volume 107, accepted the conclusion of an independent laboratory (EPL), which was that the apparent increase in squamous cell carcinomas in the head region of male Fischer rats did not result from oral exposure to paraquat. Several members of the review committee concluded, however, that topical exposure to paraquat contained in the powdered diet may have elicited tumors. The EPA committee determined that the LSR study results, coupled with negative data from the two Japanese studies (both of which used pelleted feed, hence less chance for topical exposure to dusts; see CDFA Vols. 108 and 109) suggest that protection of workers from other aspects of toxicity would also protect adequately against oncogenic hazards, since the reference dose for paraquat is relatively low (0.0045 mg/kg/day). Aldous, 7/14/89.

** **055 - 067, 010145 - 57** "Paraquat: Combined Toxicity and Carcinogenicity Study in Rats." (Woolsgrove, B. W., et al., Life Science Research, Study No: 82/ILY 217/328, 10/27/83.) Paraquat technical, 32.7% cation, administered to 70/sex/group at 150, 75, 25, 0, or 0 ppm AI in powdered feed for 113 -122 weeks with interim sacrifice of 10/sex/group at 1 year. **Possible adverse effects**: Lenticular cataracts, no NOEL; nerve degeneration, NOEL = 25 ppm; lung lesions and/or tumors, NOEL = 25 ppm. Initially reviewed as unacceptable but upgradeable with historical control data. Submission of Record # 057479 (Doc.# 205-098), historical control data for lung tumors in F344 rats, upgrades study to ACCEPTABLE status. F. Martz, 11/24/86; H. Margolis and J. Gee, 8/5/87.
NOTE: The lung lesion incidence was increased slightly in high dose males, but statistically significantly increased only in the higher two dose levels in females (dose-related). Lung lesions were clearly proliferative, but there was disagreement between reviewing pathologists as to how many were neoplasias. Dr. Martz concluded that the lung lesions, although strongly suggestive of an oncogenic effect, were not the pivotal feature of this study because: (1) lesions (or tumors) were typically late appearing and not life-shortening, and (2) the lesions appeared to be secondary to chronic inflammatory insult. The pivotal finding, according to Martz, was lenticular cataracts, with a NOEL presumed to be slightly below 25 ppm. (Note by Aldous, 6/1/92).
See also volume 233, record 212112 below for evaluation of the lungs. (Gee, 8/11/04)

053 000823 2/17/84, Summary of 010145 - 57. J. Christopher, 3/1/85.

098 057479 Addendum to 055 - 067, records 010145 - 010157 "Paraquat: Combined Toxicity and Carcinogenicity Study in Rats." Submitted with 5/4/87 rebuttal. This is a table of control incidences for pulmonary adenomas and carcinomas in F 344 rats at LSR (Suffolk or Essex). Eight studies of appropriate timeframe were provided. Range for adenomas in females was 0 to 4% (mean about 1%). No pulmonary carcinomas were found in females. Data are consistent with findings of zero pulmonary tumors in current female controls in study 055:010145, above. Margolis and Gee, 8/5/87; Aldous (no separate review), 6/1/92.

009 951015 "Paraquat: Combined Toxicity and Carcinogenicity Study in Rats." (Life Science Research, 12/77.) UNACCEPTABLE. Report consists of a protocol for 010145 - 57, followed by

a letter written in 1980 summarizing findings and reporting a **possible adverse effect** consisting of cataracts in the eyes at 150 ppm over 2 years. Sixty per sex per group were fed 0 to 150 ppm. No data other than for ophthalmoscopy. J. Christopher, 3/1/85.

091 048659 "Paraquat: Dosage Range-finding Study in Rats." (Life Science Research, Study No. 78/ILY144/111 (CTL/C/1347), 2/21/85.) Oral feed dose range finding study in rats (F344); paraquat dichloride liquid, 32.7%, w/v as cation, at 300, 200, 100, 50, 25, or 0 ppm cation in the feed for 8 weeks, to 15/sex/level; mortality and respiratory distress at 300 and 200 ppm with lung lesions at 300 -100 ppm; reduced weight gain and food consumption at 300 and 200 ppm; kidney lesions at 300 and 200 ppm; overall NOEL = 50 ppm; results used to set dose levels in combined rat study (Record #s 010145-57) at 150, 75, 25, or 0 ppm. F. Martz, 10/22/86.

107 069948 (Appendix 2 of this volume). ICI Americas discussion entitled "Oncogenic potential of paraquat - Toxicology data: Summary and overview." Key element in discussion is reference to re-examination of male F-344 rat heads by J. Ishmael (whose report is in Appendix 3, 107:069949).

107 069949 (Appendix 3 this volume). "Paraquat: Lifetime feeding study in rats - A histopathological review of slides from the head region." Re-examination of male F-344 rat heads by J. Ishmael (of ICI Central Toxicology Laboratory, Alderley Park, Cheshire, UK), who concluded that "there was no justification for combining the tumors from these separate sites [ear, nasal cavity, oral cavity, skin and subcutis of head] for assessment purposes and that the data was not consistent with a conclusion that paraquat is oncogenic." CDFA had not judged these tumors (squamous cell carcinomas from different sites in the head) to be treatment related, however EPA had concluded that "paraquat showed some evidence of carcinogenicity in the male Fischer 344 rats" in its initial review. EPA subsequently changed that conclusion after an independent review (See Appendix 1, this volume). C. Aldous, 7/11/89.

0233 - 212112 “Paraquat: Lifetime feeding study in rats: Histopathological examination of lungs.” (Ishmael, J. and M. J. Godley, ICI report CTL/P/738, 7/28/83) This report contains results of the examination of lung sections for the study in 055 - 067, records 010145 - 57, Woolsgrove *et al,* 1983, above, conducted at Life Science Research. J. Ishmael contributed to the original report but more extensive data are presented in this record. There appears to be some change in the terminology used for lung findings. Two main types of paraquat-related lesions were noted, chronic inflammation (pneumonitis) with fibrosis (increased in males at 150 ppm) and adenomatosis (significantly increased in males and females at 150 ppm). There was a suggestion of an increase in males only at 75 ppm. The incidence of adenomas and carcinomas was stated to be similar to those found in other studies using F344 rats. The NOEL for lung findings was considered to be 25 ppm, as determined in earlier reviews of this study. Supplemental information. (Gee, 8/10/04)

108 069951 "AT-5: Chronic toxicity study result - 104 week dosing study in rat." (Nippon Experimental Medical Research Institute, 3/10/82) JCL:Wistar rats, 50/sex/group, were dosed with 0, 6, 30, 100, or 300 ppm paraquat (nominal) for 2 years. Additional 6/sex/group were dosed for 26 weeks or 52 weeks for interim sacrifices. No adverse effects indicated. Apparent NOEL = 100 ppm in M and F, primarily based on RBC parameters indicating slight anemia. Serum protein levels were generally low, and kidney weights were typically reduced (without corresponding microscopic changes). Ophthalmology was performed and found negative. Study is UNACCEPTABLE, and does not appear to be upgradeable, due to lack of an apparent MTD. Other deficiencies included missing report of dosed feed analysis and lack of quality assurance documentation. C. Aldous, 7/6/89.

NOTE: EPA classified this as "core minimum" rat combined study (see "Second Peer Review of Paraquat", in CDFA Vol. 107, Appendix 1).

CHRONIC, RAT

021 951012 "Two-Year Chronic Oral Toxicity of Paraquat: Albino Rats". 7/16/64. Invalid IBT study. J. Christopher, 3/1/85.

007 014766 "Two-Year Chronic Oral Toxicity of Paraquat: Albino Rats". No lab or date given (probably summary of invalid IBT study: Record # 951012). Summary only. Thirty rats per sex per group were given 0, 50, 125 or 250 ppm for 2 years. No data. UNACCEPTABLE with insufficient information. J. Christopher, 3/1/85.

026 045131 Summary information.

CHRONIC DOG

021 951011 "Chronic Oral Toxicity of Paraquat: Beagle Dogs". 8/20/64. Invalid IBT study. J. Christopher, 3/28/85.

** **048 951014** "Paraquat: 1 Year Feeding Study in Dogs." (Imperial Chemical Industries, CTL/P/734 and 734S, 4/20/83.) Paraquat dichloride liquor (32.2%) fed to six/sex/group at 0, 15, 30 or 50 ppm for 1 year. **Possible adverse effects**: a clearly defined chronic toxicity to the lungs was reported at 30 and 50 ppm consisting of fibrosis, inflammation and alveolar cell hyperplasia. NOEL is reported to be 15 ppm or approximately 0.45 mg/kg/day for both sexes. Initially reviewed as unacceptable but upgradeable with submission of individual data. Submission of these data as Record # 057480 upgrades the study to ACCEPTABLE status. J. Christopher, 3/1/85; G. Patterson, 9/16/86; H. Margolis and J. Gee, 8/5/87.

098 057480 Individual Animal Data. Addendum to 048:951014, above.

088 045234 Partial duplicate of record # 057480.

205-175 073367 Sheppard, D. B., "Paraquat thirteen week (Dietary administration) toxicity study in beagles." Hazleton Laboratories Europe Ltd., Harrogate, England, 2/17/81. Beagle dogs, 3/sex/group, were fed 0, 7, 20, 60, or 120 ppm target dietary concentration of paraquat ion, derived from tech. test article aq. liquor (identified as Y00061/009/004, 32.2% paraquat cation). Two/sex of the 120 ppm group died between days 16 and 23. Deaths were preceded by sudden appearance of dyspnea, and often emaciation or inappetence, with some body weight losses. The surviving high dose female lost weight steadily from week 7 to 13. The major histopathology finding was alveolitis in all 120 ppm dogs, and in all 60 ppm dogs except for 1 male. Swollen cortical tubules were seen in kidneys of 1/sex at 120 ppm and in 1 male at 60 ppm: a possible treatment effect of minor importance. No DPR worksheet is needed, since the chronic dog study (Record Nos. 38955, 51120, and 54773) provides a lower NOEL. Aldous, 5/12/92.

032 951006 Cow feeding study (not reviewed).

ONCOGENICITY, MOUSE

** **083 038956** "Paraquat: Lifetime Feeding Study in the Mouse." (Imperial Chemical Industries, CTL/P/556, 6/17/81.) Paraquat, 32.7%, w/w ion; "Swiss-derived" mice, 60/sex/group (two control groups), plus 10/sex/group for 1 year sacrifice, were fed 0, 12.5, 37.5 or 100.0/125.0 ppm in the diet over 97-99 weeks; NOEL: 12.5 ppm. **Possible adverse effect**: no oncogenic effect but positive chronic toxicity in kidneys and lungs. Low survival at termination with up to 87% mortality. Initially reviewed as unacceptable, but upgradeable. Upgraded to ACCEPTABLE with submission of missing individual data, 094:051121. J. Gee, 4/17/86; H. Margolis and J. Gee, 8/5/87.

094 (3 volumes) 051121 Addendum to 083:038956. Individual Animal Data for "Paraquat: Lifetime Feeding Study in the Mouse." Submitted with 2/17/87 rebuttal. H. Margolis, 7/21/87.

017 951010 Summary of record # 038956. J. Christopher, 3/1/85.

007 014765 Duplicate of record # 951010.

109 069952 "AT-5: Chronic toxicity study result - 104 week dosing study in mouse." Nippon Experimental Medical Research Institute, 3/10/82. JCL:ICR mice, 60/sex/group, were dosed with 0, 6, 30, 100, or 300 ppm paraquat (nominal) for 2 years. An additional 10/sex/group were dosed for 26 weeks or 52 weeks for interim sacrifices. No adverse effect indicated. NOEL = 30 ppm (decreased RBC counts, Hct, and Hb content in M and F, slight decrease in white blood cell count in M and F during interim sacrifices, and slight decrease in plasma protein levels in M and F). Study is UNACCEPTABLE, and does not appear to be upgradeable, due to lack of an apparent MTD. Other deficiencies included missing report of dosed feed analysis and lack of quality assurance documentation. C. Aldous, 7/14/89.

NOTE: EPA classified this as a "supplementary" oncogenicity study (see "Second Peer Review of Paraquat", in CDFA Vol. 107, Appendix 1).

REPRODUCTION, RAT

** 083, 093, 096 038955, 051120, 054773 "Paraquat: Multigeneration Reproduction Study in Rats: Three Generations." (Imperial Chemical Industries, CTL/P/719, 12/22/82.) Paraquat, 32.7% ion; 15 males/30 females per group were fed 0, 25, 75 or 150 ppm; 3 generations, 2 litters. NOEL (reproductive) > 150 ppm; Maternal NOEL (lung injury) = 75 ppm; NOEL for adult males (focal alveolar histiocytosis) = 25 ppm. No adverse reproduction effect; the mortality rate in the high dose females due to lung injury suggests additional stress in lactating females or the increased food consumption during lactation resulted in intake of more test article to a lethal dose. The lung toxicity of paraquat has been identified in other studies. Initially evaluated as unacceptable (but upgradeable) due to lack of individual animal data. Submission of Record # 051120 and 054773 (individual data) upgrades study to ACCEPTABLE status. J. Gee, 4/16/86; H. Margolis and J. Gee, 8/5/87.

EPA one-liner: supplemental with no conclusion. Provisional Repro NOEL > 150 ppm (HDT); provisional based on lack of individual data.

093 (3 volumes, Parts 1 - 3) 051120 "Individual Animal Data (Parts I and II)" and 096 054773

"Individual Duration on Study" (Both record numbers are part of Report No. CTL/P/719S) addenda to 083:038955 "Paraquat: Multigeneration Reproduction Study in Rats: Three Generations." Addenda submitted with 12/1/86 rebuttal. H. Margolis and J. Gee, 8/5/87.

052 951024 Summary of record # 038955. J. Christopher, 3/1/85.

014 951021 Summary of record # 038955. J. Christopher, 3/1/85.

007 951023 "Paraquat: Three Generation Reproduction Study in Rats." (K. Fletcher, Imperial Chemical Industries Report No. HO/IH/P/19, 6/17/81[or 3/72]), technical grade solution with 25.85% paraquat ion, twelve males and 24 females per group were fed 0, 30 or 100 ppm. There were two litters per generation. UNACCEPTABLE, insufficient information but no adverse reproduction effect reported. Not upgradeable (26% purity of test article, no diet analysis, only two doses and the higher one is judged as too low to meet guidelines, no individual data, inadequate number of animals). J. Christopher, 3/1/85.
EPA one-liner: Supplementary. NOEL $\geq$ 100 ppm paraquat ion = 10 mg paraquat ion/kg/day.

021 046567 Summary information.

205 212111 Duplicate of record 951023. No worksheet. (Gee, 8/10/04)

REPRODUCTION, RABBIT

007 951022 "Reproduction in Paraquat-Treated Rabbits." (Imperial Chemical Industries, Report No. IHR/193, 2/66.) Paraquat dichloride, 24% w/v paraquat ion, was administered by several routes at 30 ppm, 2.4 mg/kg/day x 8 plus 1.2 x 20 or 1.2 mg/kg/day x 10 plus 12 mg/kg/day. UNACCEPTABLE, insufficient information but no adverse effect on reproduction identified. Excessive mortality (no data) at last 2 dosing schedules. Summary only. J. Christopher, 3/28/85.
EPA one-liner: Invalid.

TERATOGENICITY, RAT

** 188 119894 "Paraquat:: Developmental Toxicity Study in the Rat." (M. C. E. Hodge, ICI Central Toxicology Laboratory, Study No: RR0593, 11/30/92). Paraquat, purity (paraquat ion content) 38.2% w/v, was administered via oral gavage at doses of 0 (deionized water), 1, 3, or 8 mg paraquat ion/kg/day to 24 pregnant female Alpk:APfSD (Wistar-derived) rats /group during Days 7 through 16 of gestation. Maternal body weight gain was reduced during the first two days of dosing for the high dose group. Maternal NOEL = 3 mg paraquat ion/kg/day. No evidence of major fetal abnormalities. Developmental NOEL = 8mg paraquat ion/kg/day. ACCEPTABLE. (Kishiyama, J., and Iyer, P. (10/06/93).

** 007 951017 "Paraquat Dichloride: Teratogenicity Study in the Rat." (Imperial Chemical Industries, Report No. CTL/P/365, 6/5/78.) Thirty rats per group were given 0, 1, 5 or 10 mg/kg by gavage on days 6-15 of gestation. Only 18 in the high dose group survived and were pregnant. Mid-dose was actually 4 mg/kg by analysis of dosing solution. Maternal toxicity (mortality, lung and kidney lesions) were reported at 5 and 10 mg/kg. Fetotoxicity was associated with maternal toxicity; NOEL for both parameters was 1 mg ion/kg. Report includes all individual data and pilot study.

Histopathology on target organs of dams. No adverse effects reported. ACCEPTABLE. J. Christopher, 3/1/85.
EPA evaluated this as minimal with a NOEL (fetotoxicity) of 1 mg ion/kg; maternal NOEL: 1 mg ion/kg.

005 951016 Summary of 951017. J. Christopher, 3/1/85.

TERATOGENICITY, MOUSE

** 188 119893 "Paraquat (technical): Oral (Gavage) Mouse Developmental Toxicity Study." (K. Palmer, Toxicol Laboratories Limited, CTL Study No. RM591, November 1992). Paraquat, purity (paraquat ion content) was 38.2% w/v, administered via oral gavage at concentrations of 0 (purified water), 7.5, 15, or 25 mg paraquat ion/kg/day to 26 pregnant female Crl:CD-1(ICR)BR mice/group during days 6 through 15 of pregnancy. One female died and four others were killed in extremis. Some animals in the 25 mg/kg group exhibited symptoms of labored breathing, piloerection, hunched posture, hypothermia, hypoactivity, pale extremities and eyes and dark red lung lobes at necropsy. Maternal body weight and bodyweight gain for the high dose level also were reduced. The weight of lungs and trachea at this level increased. Additionally, an increase in fetal skeletal variations (vertebrae and hindlimb) along with a reduction in fetal body weight was observed. Maternal and Developmental NOEL = 15 mg paraquat ion/kg/day. ACCEPTABLE. (Kishiyama, J.; Iyer, P., 9/30/93).

205 - 088 045235 "Paraquat Dichloride: Teratogenicity Study in the Mouse." (Imperial Chemical Industries, CTL/P/364, 6/12/78.) Twenty to twenty-three mice were given paraquat (100% purity, batch # ADY M 76/G) at 0, 1, 5, or 10 mg/kg by oral gavage, days 6-15 of gestation and killed on day 18. No clinical signs of toxicity but reduced body weight at 5 and 10 mg/kg. No adverse effect identified. UNACCEPTABLE, upgradeable: needs justification for the dosage range selected (considering that the preliminary study appears to have indicated that a higher dosage range would have been tolerated). J. Christopher, 3/1/85 and G. Patterson, 9/16/86. Re-examined in rebuttal response by C. Aldous on 7/13/89, and again on 8/27/91 in connection with Record 089664 (below). Considered in context of EPA data gap reconciliation by Aldous, 1/18/90.
EPA considers this "Core Minimum", and negative at 10 mg ion/kg.

009 951018 Brief version of 045235. J. Christopher, 3/1/85.

205 -187 089664 Hodge, M. C. E., "Interim report to first supplement to paraquat dichloride: teratogenicity study in the mouse." ICI Central Toxicology Laboratory Report No. CTL/T/2758, 6/28/91. Report relates to study 205-088 045235, above. A 2-paragraph statement re-states previous rationale for dose selection. Supplementary data include sex distribution of fetuses by litter. No worksheet, however see rebuttal of 8/27/91 by C. Aldous.

TERATOLOGY, RABBIT

205 -185 096693 Tinston, D., Study Director, protocol entitled "Paraquat: Second teratogenicity study in the rabbit." ICI Central Toxicology Laboratory [CTL Study No. RB0547], submitted with cover letter date of 3/26/91. An earlier study had to be abandoned due to lack of sufficient pregnant females and live litters to meet EPA requirements. This second study was already in

progress as of March, 1991. Protocol was examined by J. Gee (no worksheet), 4/22/91.

MUTAGENICITY, GENE MUTATION

Bacteria

** 073 022828 "Mutagenicity Testing of Paraquat (S. typhimurium)." (Inveresk Res. Int'l., 10/77.) Ames test, paraquat 99.9% purity, Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 were tested with and without rat liver activation at 0, 10, 33, 100, 333, and 1000 ug/plate, in triplicate. Cytotoxicity at 1000 ug/plate. No adverse effect, no increase in reversion rate. ACCEPTABLE**.** J. Gee, 4/13/85.
EPA one-liner: Minimal with no adverse effect.

084 039758 "Mutational Studies with Diquat and Paraquat in vitro (S. typhimurium; Ames assay)." (Published in Mutation Res. 68: 183 (1979)). UNACCEPTABLE**. Possible adverse effect** with an increase in forward mutation rate in Salmonella reported in G46, TA92 and TA1535 for 8-azaguanine resistance, with 3 plates per concentration at 0 - 1.0 ug/plate. The article also reports negative findings in his reversion with toxicity at > 10 ug/plate. Although the publication is inadequate, a genotoxic effect has been identified and must be evaluated in the light of other studies on paraquat. J. Gee, 4/16/86.

007 951025 "Paraquat Mutagenic Potential in S. typhimurium Mutagenicity Assay." (Lab. name not specified, Report No. CTL/P/243, 5/76.) Salmonella strains TA1535, TA1538, TA98 and TA100 were tested at 0, 0.16, 0.80, 4.00, 20.00, 100, 500, 2500 and 5000 ug/plate in duplicate +/- S9 (rat liver induced with Aroclor and phenobarbital). No adverse effects identified. UNACCEPTABLE**,** no individual plate counts, no positive controls for non-activation plates. Upgradeable. J. Christopher, 3/1/85.
EPA one-liner: Minimal with no effect.

205 212133 “Mutagenicity testing on paraquat dichloride in microbial systems.” (Shirasu, Y., M. Moriya and T. Ohta, Institute of Environmental Toxicology, 1978) Five strains of *Salmonella typhimurium*, TA1535, TA1537, TA1538, TA98 and TA100, were exposed to paraquat dichloride (100%) with and without rat liver activation. Also included was *E. coli* WP2 hcr. The ug/plate were 0.5, 1, 5, 10, 50, 100 and 500 ug, in duplicate, one trial. There was no increase in revertants. Paraquat was toxic at 50 ug/plate without activation and at 100 ug/plate with activation. Positive controls were functional. Unacceptable (summary report). No adverse effect. No worksheet. (Gee, 8/11/04)

205 212133 “Mutagenicity testing on paraquat dichloride in microbial systems.” (Shirasu, Y., M. Moriya and T. Ohta, Institute of Environmental Toxicology, 1978) Host mediated assay in ICR male mice with mice exposed to 0 (water), 2 x 5 mg/kg or 2 x 20 mg/kg paraquat (100%) or the positive control, dimethylnitrosamine, single dose. There were six per group with doses given 24 hours apart by oral gavage. Immediately following the second dose, mice were given an ip injection of *Salmonella typhimurium* G46 in 2 mls at 7 x 10^{8} per ml. Three hours later, the mice were sacrificed and the peritoneal fluid removed and plated in triplicate for revertants. There was no increase in revertants. The positive control was functional. Unacceptable (summary report). No worksheet. No adverse effect. (Gee, 8/11/04)

Mammalian cells

082 037210 "Paraquat Dichloride (Technical Liquor): Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells." (ICI, report # CTL/P/1398, 9/24/85) Mouse lymphoma; paraquat dichloride (technical liquor, 45.66% purity) with 53.52% water was used; 0, 31.25, 62.50, 125, 250, 500 or 1000 ug/ml, 2 hours, +/-S9; TFT to select; dilution and count negative-growth wells; Five trials with inconsistent results. No consistent increase in mutation frequency. No adverse effect. UNACCEPTABLE, not upgradeable. Protocol differs from usual: cells were treated for 2 hours and tested immediately for survival. Remainder were grown for 48 hour expression time, then diluted to give 2 x 10^3 per well. Count negative, no growth wells. (J. Gee, 2/7/86).

** 082 037213 "Paraquat Dichloride (Technical Liquor): Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells." (ICI, report # CTL/P/1374, 9/17/85), Mouse Lymphoma; paraquat dichloride (analytical, 99.6%) 0-1004 ug/ml, 2 hours, +/- S9 from rat liver; TFT to select, 48 and 72 hour expression; six trials. No consistent increase in mutation frequency; cytotoxic at higher concentrations. No adverse effect. ACCEPTABLE. See 037210 for similar results. (J. Gee 2/7/86)

See "COMMENTS RELATING TO STUDY TYPES REQUIRED BY SB-950", following the "Neurotoxicity" section of this summary, for an overall assessment of gene mutation studies.

MUTAGENICITY, CHROMOSOME

**** 082 037212** "Paraquat Dichloride: A Cytogenetic Study in Human Lymphocytes In Vitro." (ICI, report # CTL/P/1351, 9/3/85) Paraquat dichloride (99.6% in saline) was tested at 0, 125, 1250, 2500 and 3500 ug/ml without S9 and 0, 350, 1750, 2500 and 350ug/ml with S9; 3 hours; PHA to stimulate. 100 cells per culture were scored, 2 cultures per concentration were analyzed. **Possible adverse effect**. Positive for increase in % abnormal cells +/- S9 in both donor cultures. ACCEPTABLE. Note: an increase in SCE's in Chinese hamster cells in vitro has also been reported - see 082 - 037215. (J. Gee, 2/10/86)

073 022829 "Paraquat: A Cytogenetic Study in the Rat." (ICI, report # CTL/P/367, 7/5/78), Chromosome aberrations in vivo in rats. Paraquat 100% purity, batch ADY M 76/G. Six to 8 male rats were given 0, 6.5, 12.5 or 19.0 mg/kg by oral gavage daily for five days and sacrificed 6 hours after the last dosing. Dose selection was based on the LD50. Fifty cells/animal were scored - some animals had fewer cells scored. No adverse effect on chromosomes reported. UNACCEPTABLE, not upgradeable. No evidence the MTD was used. Also, Schmid protocol is not acceptable by guidelines. Problem with staining chromosomes from paraquat-treated animals made quantitation of gaps and breaks more difficult. (J. Gee, 9/13/85)
EPA one-liner: acceptable with negative results up to 19.0 mg/kg.

007 951027 "Paraquat: Further Cytogenetic Studies in the Rat." (ICI, report # CTL/P/442, 6/13/79), chromosome aberrations in rats. A series of tests in rats are reported studying the effect of paraquat on metaphase chromosomes and how it alters staining properties of Giemsa for chromosomes. No adverse effect reported. UNACCEPTABLE, information supplemental. (J. Christopher, 3/4/85)

**** 082 037215** "Paraquat Dichloride: An In Vitro Sister Chromatid Exchange Study in Chinese Hamster Lung Fibroblasts." (ICI, report # CTL/P/1392, 9/24/85), Chinese hamster SCE in vitro; paraquat dichloride (99.4%), 0 -124 ug/ml without S9 and 0 - 245 ug/ml with S9; 3 hours treatment time. Increased incidence of SCE's per cell and per chromosome in +/- S9. Cultures without S9 were more affected, possibly due to protein binding of paraquat. Mitotic indices were also reduced. ACCEPTABLE with **possible adverse effect**. (J. Gee, 2/10/86)

082 037214 "Evaluation of Paraquat Dichloride (technical) in Mouse Micronucleus Test." (ICI, report # CTL/P/1369, 9/4/85) Micronucleus; paraquat dichloride (technical 33.07% ion) at 0, 51.75 and 82.80 mg/kg in a single dose by oral gavage to 5/sex/group sacrificed at 24, 48, and 72 hours. No increase in frequency of micronuclei in PCE's or % PCE's reported. No adverse effect on micronucleus formation. UNACCEPTABLE, not upgradeable (dose levels too low). Due to a calculation error, doses used were lower than intended. This test depends upon dosing in the toxic range. (J. Gee, 2/10/86)

084 039760 "Mutational Studies with Diquat and Paraquat In Vitro." (journal article in Mutation Res. 68:183-193 (1979). UNACCEPTABLE. **Possible adverse effect**. Positive effect in recessive lethals in Aspergillus nidulans reported after treatment for 0, 2 and 4 hours at 20 mg/ml. Percent recessive lethals in conidia in controls, 0.24, and 5.00 at 4 hours. Difficult to evaluate significance. (J. Gee, 4/16/86)

007 951026 "Paraquat: Dominant Lethal Study in Mice." (Inveresk Res., no date), No information on purity of test article, fifteen males were treated with 0.04, 0.40 or 4.00 mg/kg/day for 5 days orally (by gavage ?), 30 for solvent controls, EMS and cyclophosphamide as positive controls in CD-1 mice. Males were mated 1:2 with females. No signs that MTD was approached - no signs of toxicity reported. No decrease in fertility or pre-implantation loses, and no increase in early fetal deaths noted. No adverse effect indicated. Report contains a discussion of the LD50 determination. UNACCEPTABLE (insufficient information), upgradeable. (J. Christopher, 3/4/85)

098 057485 "Paraquat Dichloride (Technical): An Acute Cytogenetic Study in the Rat" (ICI Central Toxicology Laboratory, UK, report # CTL/P/1560, 3/26/87), paraquat dichloride (technical) 33.07% w/w paraquat ion, lot # 460, grade 6219, administered via gavage at doses of 0, 15, 75, and 150 mg/kg. Chromosome preparations made from bone marrow cells extracted 12, 24 or 48 hours after dosing. **Adverse effect**: increase in aberrations at 75 and 150 mg/kg in females at 24 hours but not at 12 or 48 hours; slight reduction in mitotic index at 24 and 48 hours. NOT ACCEPTABLE, upgradeable with submission of historical control data. (H. Margolis and J. Gee 8/5/87).

110 069954 Virtual duplicate of 098:057485. No new CDFA action required. J. Gee, 7/11/89.

DNA Effects

** 082, 098 037211, 057481 "Paraquat Dichloride: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures." (ICI, report # CTL/P/1339, 9/4/85), rat hepatocytes UDS; paraquat dichloride (99.6%), 10^{-2} to 10^{-8} M; 19 hours plus 18 hours chase. Primary rat hepatocytes were isolated from male rats. Counted 50 cells/slide, three slides per dose level, two experiments using different rat. No UDS reported. ACCEPTABLE. (J. Gee, 2/7/86)

084 039759 "Mutational Studies with Diquat and Paraquat In Vitro (UDS in Human Epithelial-like Cells)." (Publ. in Mutation Research 68:183 (1979)). DNA repair in Salmonella. Exposure to 20, 100, 1000 or 2000 mg/ml (2000 was cytotoxic). **Possible adverse effect**: A positive effect on growth inhibition of Salmonella uvrB versus uvr+ was reported and an increase in the grains/nucleus in human epithelioid cells (EUE). This is in contrast to the study on rat hepatocytes above. UNACCEPTABLE (summary information only). (J. Gee, 4/16/86)
Note: the molecular weight of paraquat is 186.25. At 10^{-2}M, this is equivalent to 1860 mg/ml so the two studies covered similar ranges in concentration. The explanation for the difference in response between the rat hepatocytes and the human cells is not clear.

** 098 057482 "Paraquat Dichloride (Technical): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo." (ICI Central Toxicology Lab., UK., study # SR0214, 3/31/87). Technical Paraquat Dichloride, lot # 460, grade: 6219, 33.07% paraquat ion. Administered by gavage to 2-5 male rats/group at 0, 45, 75, and 120 mg/kg in 4 separate experiments, 2 for each of 2 exposure periods, 4 hours and 12 hours. Isolated hepatocytes were incubated for 4 hours with 3H-thymidine followed by an overnight chase with unlabeled thymidine. Three slides per animal. Net grains determined by autoradiography. No adverse effect. ACCEPTABLE. (H. Margolis and J. Gee, 8/5/87)

110 069953 Virtual duplicate of 098:057482. No new CDFA action required. J. Gee, 7/11/89.

226 - 063 062174 [Diquat data] Published article citing another study in which paraquat was supposedly found negative for cytogenetic effects on the bone marrow of mice. There were no data for evaluation. C. Aldous, 7/89.

205 212133 “Mutagenicity testing on paraquat dichloride in microbial systems.” (Shirasu, Y., M. Moriya and T. Ohta, Institute of Environmental Toxicology, 1978) Rec assay with *Bacillus subtilis* strains H17 (wild type) and M45 (recombination deficient) using paraquat (100 % purity) on a filter disk. The ug/disk, in duplicate, were 0 (water), 20, 100, 200 or 500. Kanamycin and mitomycin C were the positive controls. The length of the inhibition zone was measured after an overnight incubation. From the table, apparently a second assay was performed at lower concentrations, being 0, 1, 5, 10, 50 and 100 ug/disk, single culture. No activation was included. There was no difference in the zone of inhibition between the two strains with paraquat. However, the positive controls functioned as expected with no difference with kanamycin and a significant difference with mitomycin C. Unacceptable (no activation included, single culture). No adverse effect. No worksheet. (Gee, 8/11/04).

NEUROTOXICITY

Not required at this time.

COMMENTS RELATING TO STUDY TYPES REQUIRED BY SB-950

In summary, chronic effects, especially in lungs, kidneys and eyes, have been reported in rats, dogs and mice. There is evidence for an oncogenic effect in rats in the reports on file and reviewed as of this date. Lung adenomas and carcinomas were significantly increased in female rats but the

diagnoses of many of these lesions differed between several pathologists such that a clear conclusion of oncogenicity remains to be shown. No reproductive or teratogenic effect in rats and mice in the absence of maternal toxicity has been identified.

There is some evidence of genotoxic effects. Two studies on his⁻ reverse mutation in Salmonella were negative while a positive effect in a forward mutation to azaguanine resistance in Salmonella is reported in a publication. This test system, however, does not have an extensive data base so the meaning of the positive response is difficult to evaluate. Two other studies on mammalian cell mutation in L5178Y TK+/- were negative. The weight of evidence is against gene mutations as measured by frameshift or base pair-change mechanisms.

Two reports of in vitro studies on chromosome effects were positive, one in human lymphocytes for aberrations and one in Chinese hamster cells for sister chromatid exchanges. This suggests a clastogenic effect of paraquat. One in vivo study in rats found no increase in aberrations but the dose may not have been adequate or the bone marrow may not have been exposed to the chemical. A second in vivo cytogenetics study in rats was also negative at a dose which did have a slight effect on the mitotic index but the bone marrow may not be a target tissue. The mouse micronucleus test was negative but an inadequate dose was used so the result is a no-test. A mouse dominant lethal test was also negative but, again, the doses used are in question. The other positive response in this test area was an increase in recessive lethals in Aspergillus nidulans. This is not a very common test and relevance to human effects is not clear.

In the third area of mutagenicity testing, two reports on induction of unscheduled DNA synthesis do not agree. Negative results were obtained in primary rat hepatocytes in a good study while positive increases in grains/nucleus are reported in a publication for human epithelioid cells over the same concentration range. The publication does not contain enough information for evaluation other than the table of data presented. This same publication also contained the information that a growth differential was seen in Salmonella strains uvrB versus uvr^+, indicating, again, an effect on DNA damage/repair systems. Because of the nature of the information in the publication, this difference cannot be resolved and a genotoxic effect cannot be ruled out.

Thus, there is some evidence in two of the three test areas for genotoxicity. It should be remembered that carcinogenicity is not the only endpoint of interest for genotoxicity. The meaning of positive effects in this area still needs clarification from further research and correlation between such effects and animal/human endpoints. J. Gee, 4/86.

MISCELLANEOUS STUDIES:

205 - 175 073365 Hardy, C. J., et al. "Assessment of accumulation of paraquat in the lungs: 3 week inhalation study in rats (15 exposures)." Huntingdon Research Centre, Cambridgeshire, 8/22/80. Technical liquor was stated to be about 40% paraquat ion. Material was nebulized to mean concentrations of 0.014, 0.106, or 0.532 mg paraquat ion/l (all particles were in respirable range). Each exposure episode was 6 hr. Total numbers of young (114-146 g) CD rats/group for all studies below were 10/0 (M/F) for controls, 85/10 (M/F) for low dose, 85/10 (M/F) for medium dose, and 85/10 (M/F) for high dose. **Accumulation studies:** protocol was to sacrifice five males/group after 1, 2, 3, 4, 5, 10, or 15 exposures (1 exposure per consecutive weekday). In addition, 5 females/group were to be killed after 5 or 15 exposures to evaluate possible sex-specificity [none was found]. (Actual numbers killed in high dose groups were reduced due to

on-study deaths). Analyses were to evaluate concentrations of paraquat in lungs and kidneys after varying lengths of time of repeated exposure. [No paraquat was detected in kidneys, even at the high dose level]. **Elimination studies:** Rats were killed at intervals after either (1) a single exposure or (2) 15 consecutive weekday exposures: intervals after the single or final exposure were 7 hr or 1, 2, 3, or 6 days. The purpose of this study was to evaluate half-life in lungs. **Results**: The high dose led to deaths of 13 males and 1 female, typically on days 6 and 7. Deaths were often preceded by pilo-erection, rapid breathing, and brown stains around the nose. Lung concentrations of paraquat in the mid-dose group plateaued at about 1.3 mg paraquat/g tissue after the third exposure. Lung concentrations in the high dose group peaked on about day 4 (4.71 mg paraquat/l), dropping to about half that concentration with continued dosing. Mean lung weights increased sharply after the fifth exposure in the high dose group to about 170% of controls. The elimination half-life was about 2 days. This is a useful disposition study. Aldous, 5/13/92.

205 - 175 073366 Grimshaw, P. et al., "Three week inhalation study in rats exposed to an aerosol of paraquat (Repeat Study)." Huntingdon Research Centre, Ltd., Cambridgeshire, Dec. 6, 1979. Groups of 4 S-D rats/sex/group were dosed once or three times (on consecutive weekdays) for 6 hr/treatment to doses of 0, 0.01, or 0.1 mg paraquat/l. Body weights and clinical signs were monitored for some rats over 3 weeks of treatment, however histological examinations of rats were limited to the above treatment intervals. Histological examinations were limited to nasal passages, pharynx, larynx, and lungs. Body weights and clinical signs were unaffected by treatment at any time in the study. The only treatment-related histological findings were limited to larynges. Single exposures led to squamous metaplasia and/or hyperplasia, predominantly at the base of the epiglottis. Three exposures led to ulceration, often with necrosis, inflammatory cell infiltration, and often adjacent squamous metaplasia and/or hyperplasia at the base of the epiglottis or at the arytenoid projections. Study provides useful supplementary data, but by its nature does not fill a data gap. Aldous, 5/14/92.

205-0234 212123 “AT-5: Subacute Toxicity Study in Mouse” (K. Maita, T. Saito, S. Tsuda, Y. Shirasu; Toxicology Division, The Institute of Environmental Toxicology; Project No. CTL/C/1866; 12/18/80) Twenty ICR-CRJ mice/sex/group received 0, 10, 30, 100 or 300 ppm of AT-5 (purity: 93.3%) in the diet for 13 weeks ((M) 0, 1.18, 3.65, 11.5, 35.8 mg/kg/day, (F) 0, 1.38, 3.91, 13.8, 41.9 mg/kg/day). Two females died in the 300 ppm group, one during week 2 and the other during week 11. Both animals exhibited pulmonary edema in the histopathological examination. The mean body weights of both sexes in the 300 ppm treatment group were lower than those of the controls at various time points during the treatment period ($p<0.05$). There was no apparent treatment-related effect upon food or water consumption, hematology, clinical chemistry or urinalysis. The mean absolute and relative liver weights of the 300 ppm males were less than those of the controls ($p<0.01$ and 0.05, respectively). The mean absolute and relative spleen and kidney weights of the 300 ppm females were greater than those of the controls ($p<0.05$, 0.01 or 0.001). The mean absolute and relative ovary weights for the 300 ppm females were less than those of the controls ($p<0.05$). However, no correlation of a microscopic lesion was apparent for any of these organs in the histopathological examination. The mean absolute and/or relative lung weights for both sexes in the 300 ppm group were greater than those of the controls ($p<0.05$ or 0.01). In the lungs of the 300 ppm group, the incidence of edema ((M) 0: 0/20 vs. 300: 3/20, (F) 0: 0/20 vs. 300: 2/20) and alveolar epithelium eosinophilic swelling ((M) 0: 0/20 vs. 300: 17/20, (F) 0: 0/20 vs. 300: 13/20) was evident. In the testes of the 300 ppm males, the incidence of circumscribed seminiferous tubule atrophy (0: 0/20 vs. 300: 2/20) was noted. **Possible adverse effect:** pulmonary lesions. **Subchronic NOEL:** (M/F) 100 ppm ((M) 11.5 mg/kg/day, (F) 13.8 mg/kg/day) (based upon the incidence of pulmonary lesions in the 300 ppm treatment group); **Study acceptable.** (Moore, 8/6/04)

205 - 0235 212126 “Report on Subacute Toxicity of AT-5 in Rats.” (K. Maito, T. Saito, S. Tsuda, Y. Shirasu; Toxicology Division, The Institute of Environmental Toxicology; Project No. C2.2/11; 12/19/80) Twenty Fischer 344 rats/sex/group received 0, 10, 30, 100 or 300 ppm of AT-5 (purity: 93.3%) in the diet for 13 weeks ((M) 0, 0.68, 1.99, 6.55, 19.6 mg/kg/day, (F) 0, 0.72, 2.11, 7.10, 21.1 mg/kg/day). No deaths resulted from the treatment. The mean body weights of both sexes in the 300 ppm treatment group were less than those of the control group over the course of the study ($p<0.001$). The mean food consumption of both sexes in the 300 ppm group was generally less than that of the control group throughout the study. No treatment-related effect was apparent in either the hematology evaluation or urinalysis. In the clinical chemistry evaluation, the mean serum LDH activity level for the 300 ppm males was greater than that of the controls ($p<0.001$). The mean serum calcium levels for both sexes in the 300 ppm group were less than those of the controls ($p<0.01$ or 0.001). The mean albumin concentration of the 300 ppm females and the globulin concentrations of both sexes in the 300 ppm group were less than those of the controls ($p<0.01$ or 0.001). In the necropsy examination, the mean relative adrenal and testes weights were greater than those of the controls ($p<0.001$). The mean absolute kidney weight for the 300 ppm males was less than that of the controls ($p<0.001$), but the mean relative kidney weights for both sexes in the 300 ppm group were greater than those of the controls ($p<0.05$ or 0.01). The mean relative ovary and spleen weights of the 300 ppm females were greater than those of the controls ($p<0.05$ and 0.001). The mean relative lung weights for both sexes in the 300 ppm group were greater than those of the controls ($p<0.01$ or 0.001). In the histopathological examination, the lungs were the apparent target organ with alveolar epithelial hypertrophy evident in the 300 ppm males (0: 0/20 vs. 300: 6/20). A subpleural lymphoid hyperplasia was noted for the 300 ppm females (0: 0/20 vs. 300: 2/20). Otherwise, a greater incidence of increased brown pigmentation was noted in the spleen of the 300 ppm female (0: 3/20 vs. 300: 9/20). **Possible adverse effect:** pulmonary lesions; **Subchronic NOEL:** (M/F) 100 ppm ((M) 6.55 mg/kg/day, (F) 7.10 mg/kg/day) (based upon the incidence of pulmonary lesions in the 300 ppm males and a greater incidence of increased brown pigmentation in the spleen of the 300 ppm females). **Study acceptable.** (Moore, 8/6/04)